(12) UK Patent Application (19) GB (11) 2 348 886 (13) A

(43) Date of A Publication 18.10.2000

(21) Application No 0006716.5

(22) Date of Filing 20.03.2000

(30) Priority Data
(31) 11073915 (32) 18.03.1999 (33) JP

(71) Applicant(s)
Asahi Kogaku Kogyo Kabushiki Kaisha
(Incorporated in Japan)
36-9 Maenocho 2-chome, Itabashi-ku, Tokyo, Japan

(72) Inventor(s)
Akira Yamamoto
Yae Kurosawa

(74) Agent and/or Address for Service
Ablett & Stebbing
Caparo House, 101-103 Baker Street, LONDON,
W1M 1FD, United Kingdom

(51) INT CL[7]
C12N 7/02 , A61K 39/12 39/145 , A61P 31/12 31/16

(52) UK CL (Edition R)
C6F FJ
C3H HF2AB HF2AC

(56) Documents Cited
EP 0337123 A WO 00/09671 A
US/EG BILLETIN 2156, BIO-RAD LABS., 1996,
P GAGNON ET AL BIOCHIM BIOPHYS ACTA, 1982,
165-173, M YAMAKI ET AL J VIROLOGY, 1980, 35,
592-602, J LUKA ET AL LIT611 REV C, MACRO-PREP
CERAMIC HYDROXYAPAATITE INSTRUCTION
MANUAL, BIO-RAD LABS.

(58) Field of Search
ONLINE: EPODOC WPI JAPIO BIOSIS CAPLUS

(54) Abstract Title
Virus or viral antigen purification method and vaccine producing method

(57) A method for purifying a virus or viral antigen is disclosed, by which a virus or viral antigen can easily and quickly be separated and purified from a culture solution or cellular emulsion containing the virus or viral antigen without lowering the activation of the virus or viral antigen. The purification method is characterised by comprising a process in that a sample containing a virus or viral antigen is contacted with hydroxyapatite as a solid carrier which is sintered at 400 through 1300°C and capable of absorbing a virus or viral antigen, and a process in that the virus or viral antigen absorbed by the solid carrier is eluted by using an eluant in a neutral pH range. In this case, it is preferable that the hydroxyapatite is formed of spherical porous particles. It is also preferable that a phosphoric acid-based buffer solution is used as an eluate. Further, a vaccine producing method using such a purification method is also disclosed.

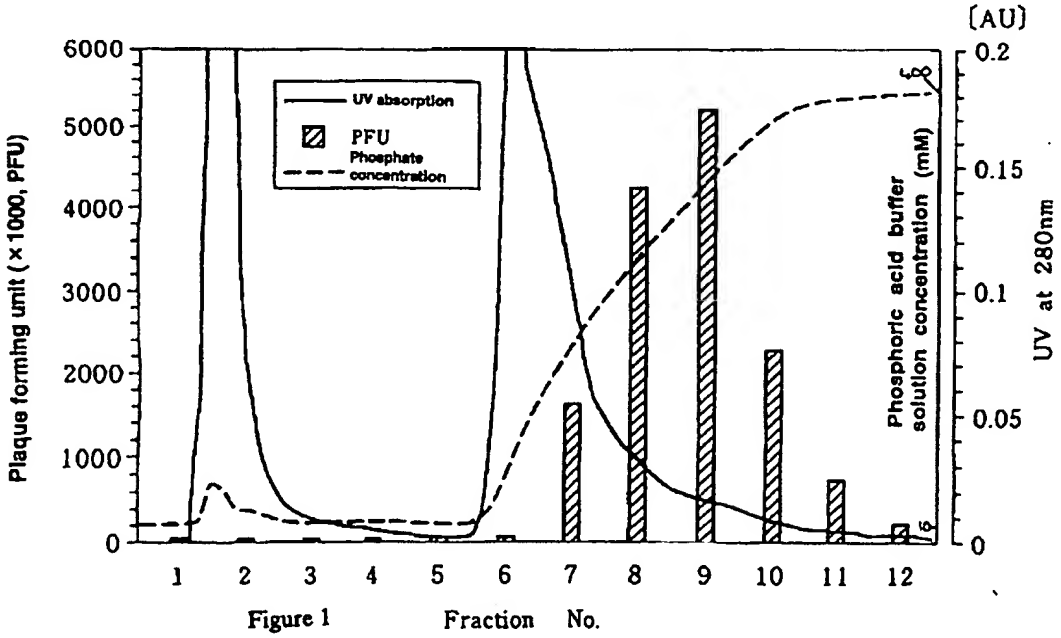

Figure 1

GB 2 348 886 A

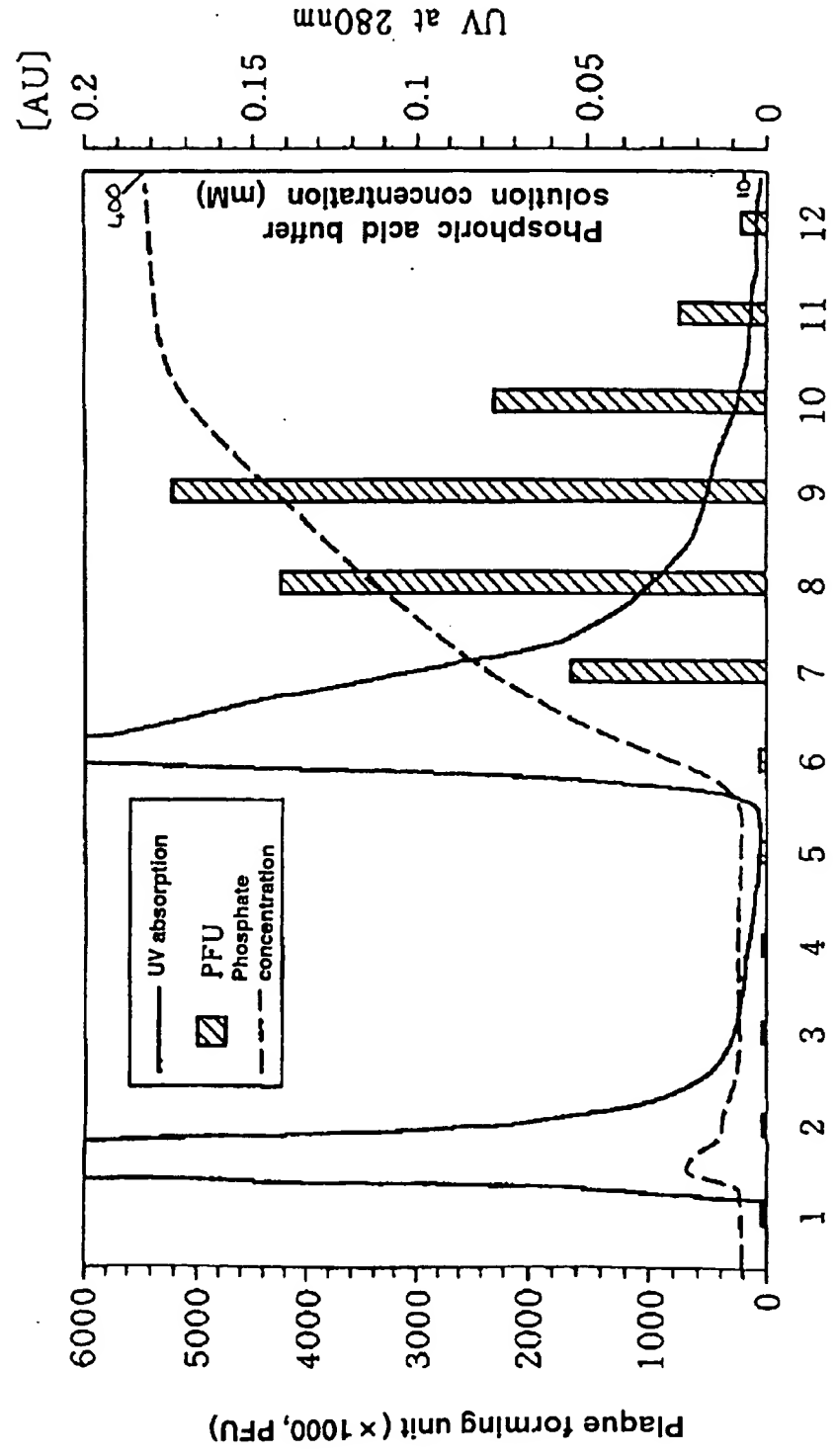
Plaque forming unit (× 1000, PFU)
6000
5000
4000
3000
2000
1000
0
UV absorption
PFU
Phosphate concentration
Phosphoric acid buffer solution concentration (mM)
80
10
UV at 280nm
[AU]
0.2
0.15
0.1
0.05
0
1
2
3
4
5
6
7
8
9
10
11
12
Fraction No.

Figure 1

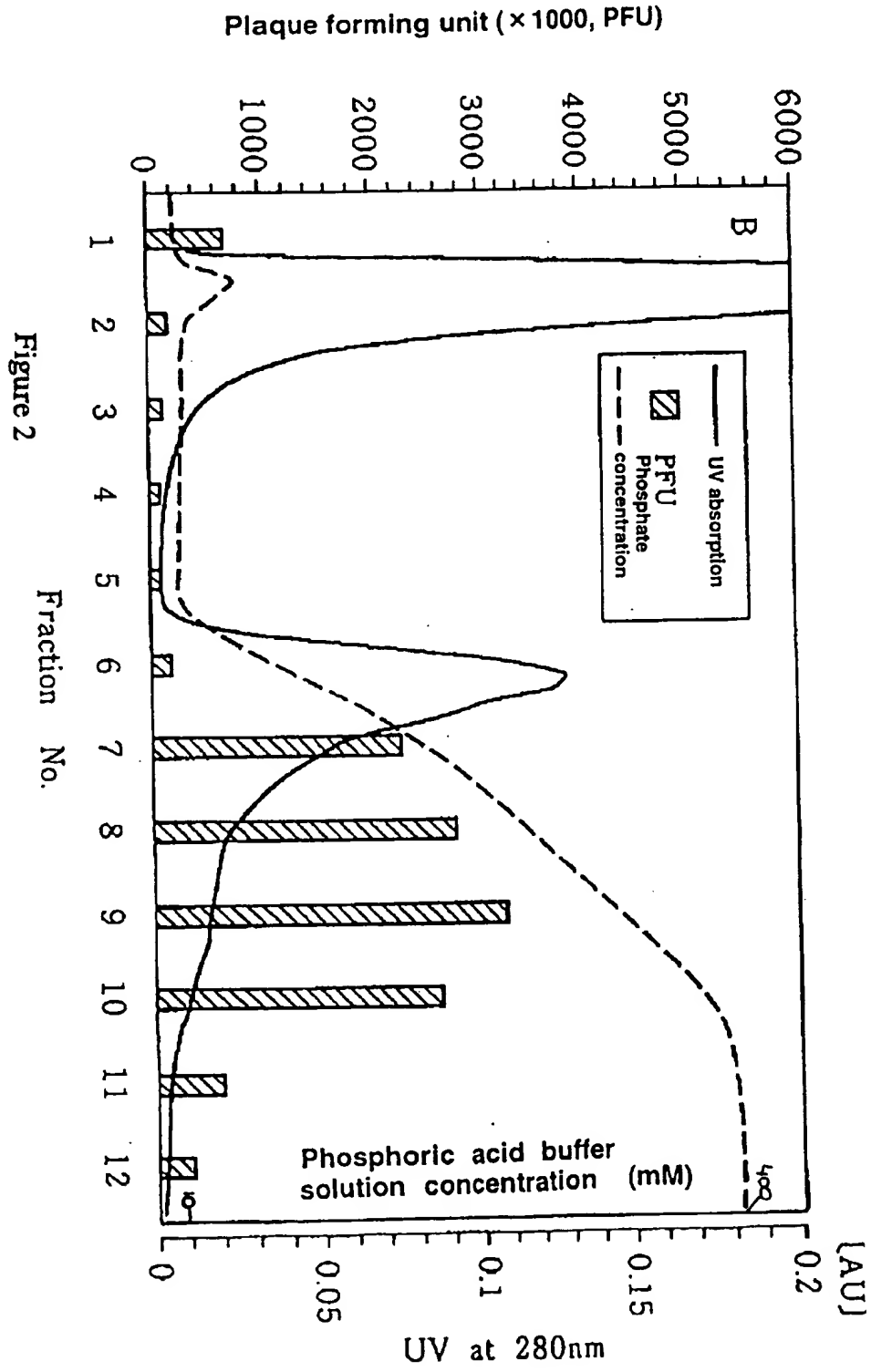
Plaque forming unit (×1000, PFU)
0
1000
2000
3000
4000
5000
6000
B
UV absorption
PFU
Phosphate concentration
Phosphoric acid buffer solution concentration (mM)
40
5
1 2 3 4 5 6 7 8 9 10 11 12
Fraction No.
0
0.05
0.1
0.15
0.2
[AU]
UV at 280nm

Figure 2

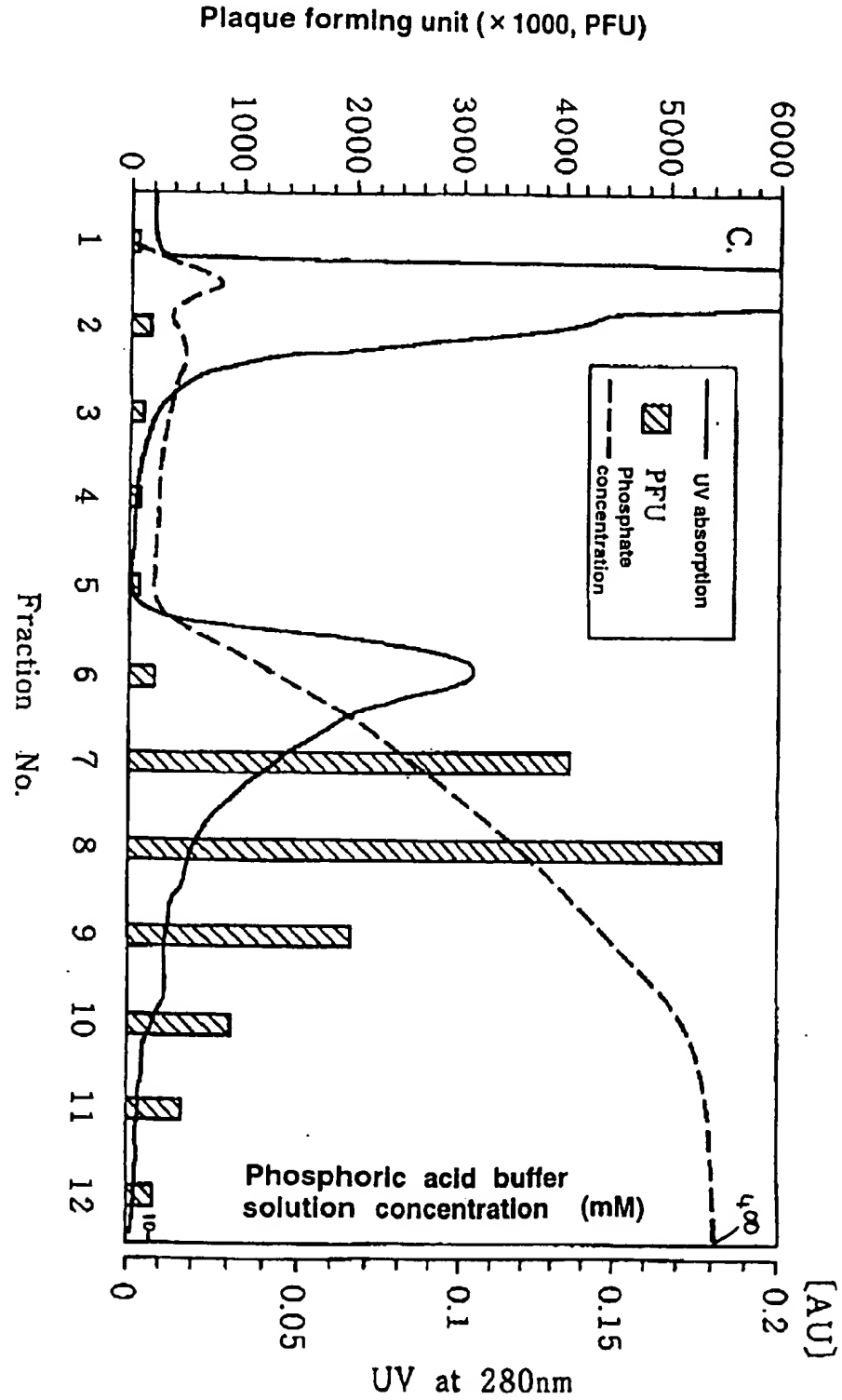
Plaque forming unit (× 1000, PFU)
0
1000
2000
3000
4000
5000
6000
C.
UV absorption
PFU
Phosphate concentration
Fraction No.
1
2
3
4
5
6
7
8
9
10
11
12
Phosphoric acid buffer solution concentration (mM)
4.8
1.0
0
0.05
0.1
0.15
0.2
[AU]
UV at 280nm

Figure 3

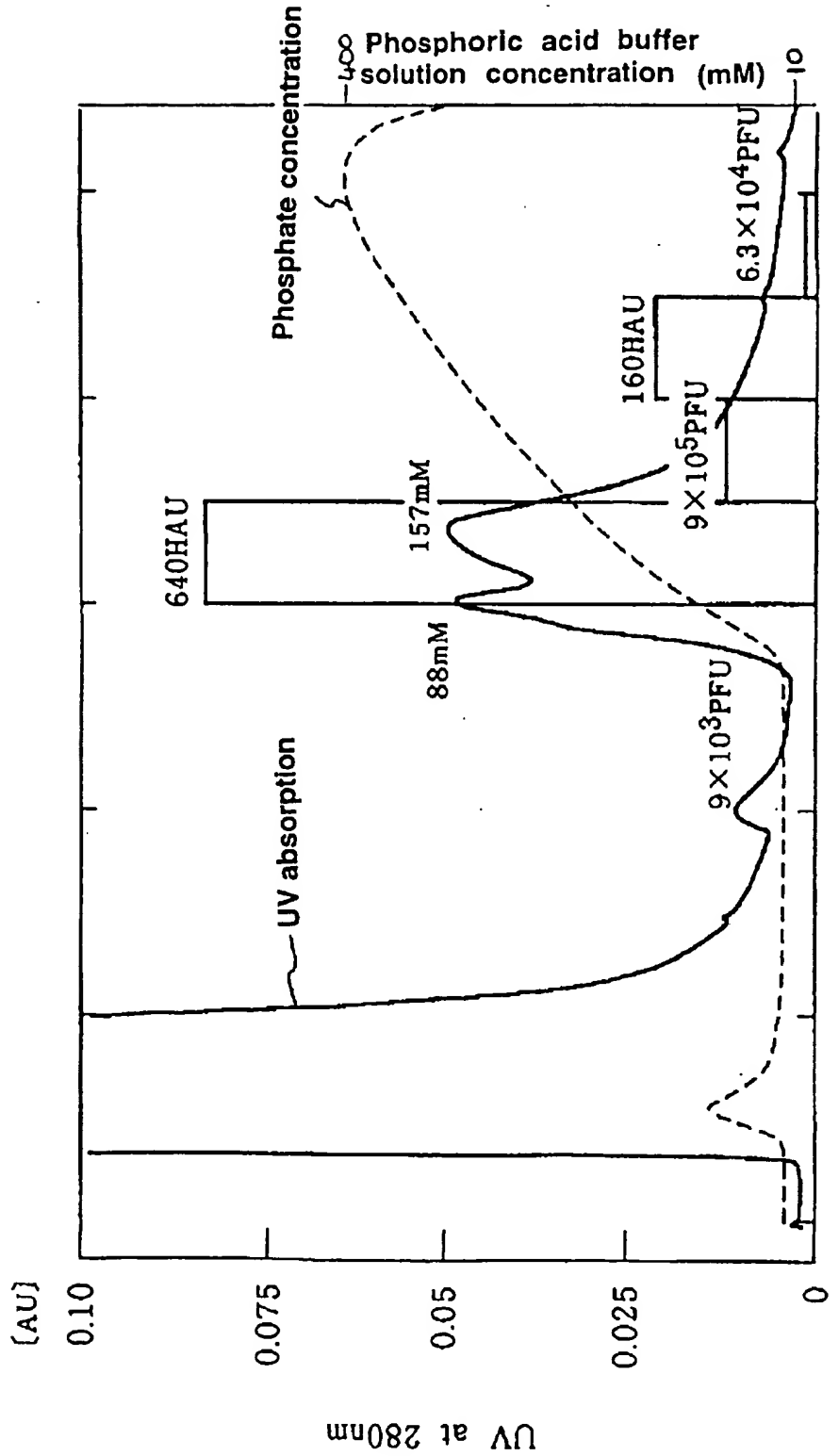
Phosphoric acid buffer solution concentration (mM)
400
10
Phosphate concentration
UV absorption
640HAU
157mM
88mM
160HAU
9×10^5PFU
6.3×10^4PFU
9×10^3PFU
fr1
fr2
fr3
fr4
fr5
UV at 280nm
[AU]
0.10
0.075
0.05
0.025
0

Figure 4

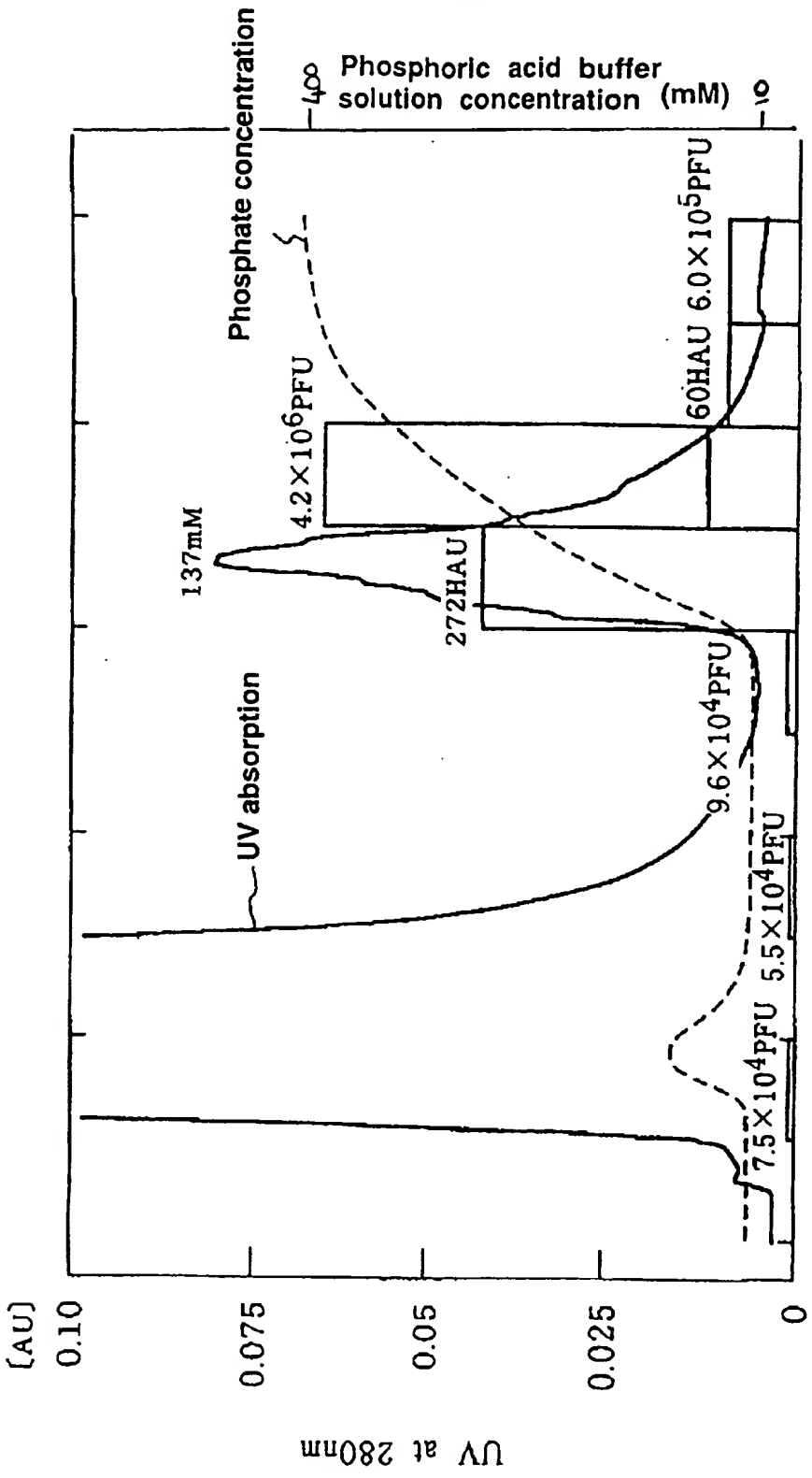
Phosphoric acid buffer solution concentration (mM)
400
0
Phosphate concentration
UV absorption
UV at 280nm
(AU)
0.10
0.075
0.05
0.025
0
137mM
4.2×10⁶PFU
272HAU
9.6×10⁴PFU
7.5×10⁴PFU
5.5×10⁴PFU
60HAU 6.0×10⁵PFU
fr1
fr2
fr3
fr4
fr5

Figure 5

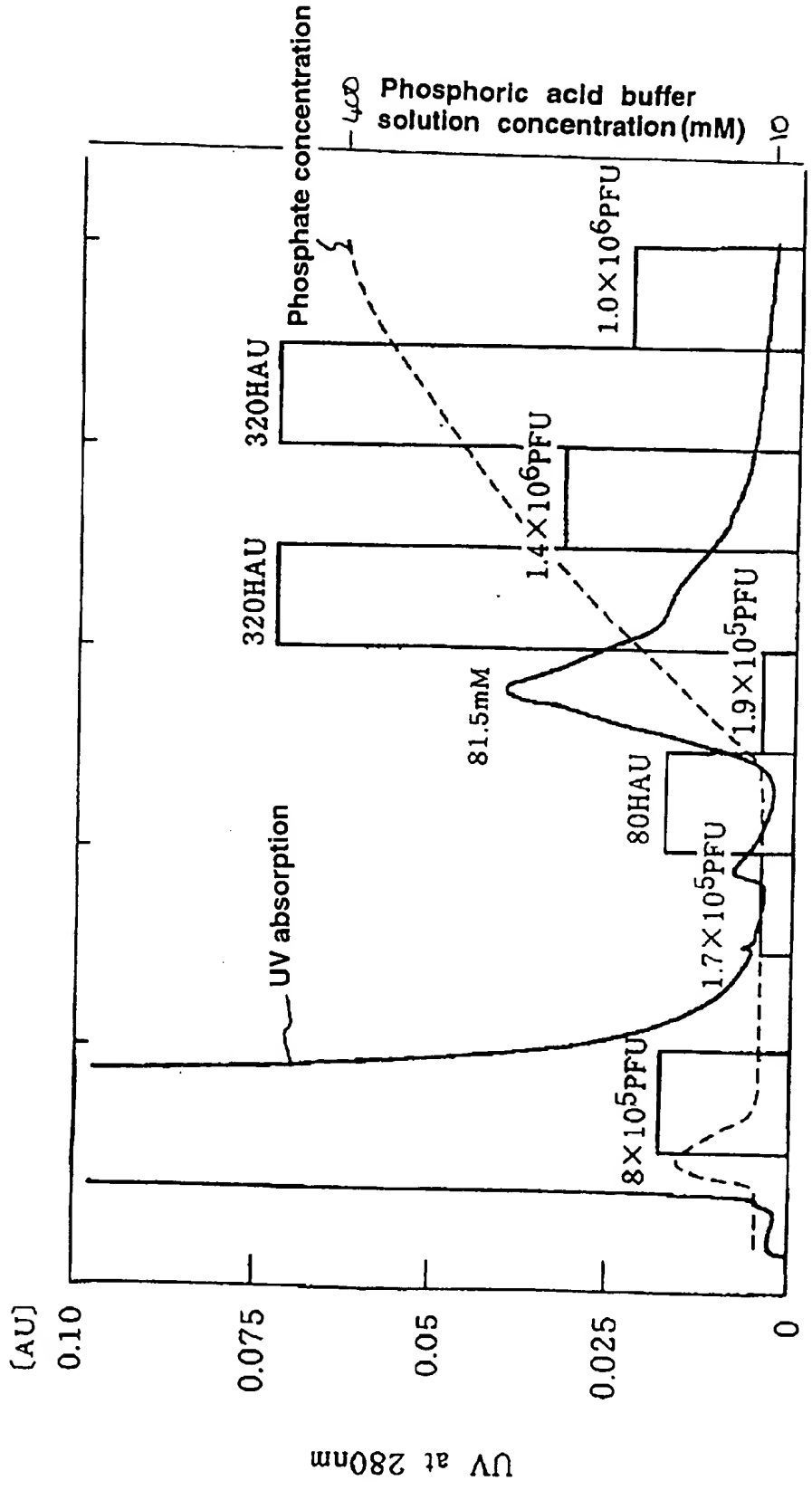
UV at 280nm
[AU]
0.10
0.075
0.05
0.025
0
UV absorption
Phosphate concentration
Phosphoric acid buffer solution concentration (mM)
400
0
81.5mM
320HAU
320HAU
80HAU
8×10⁵PFU
1.7×10⁵PFU
1.4×10⁶PFU
1.9×10⁵PFU
1.0×10⁶PFU
fr1
fr2
fr3
fr4
fr5

Figure 6

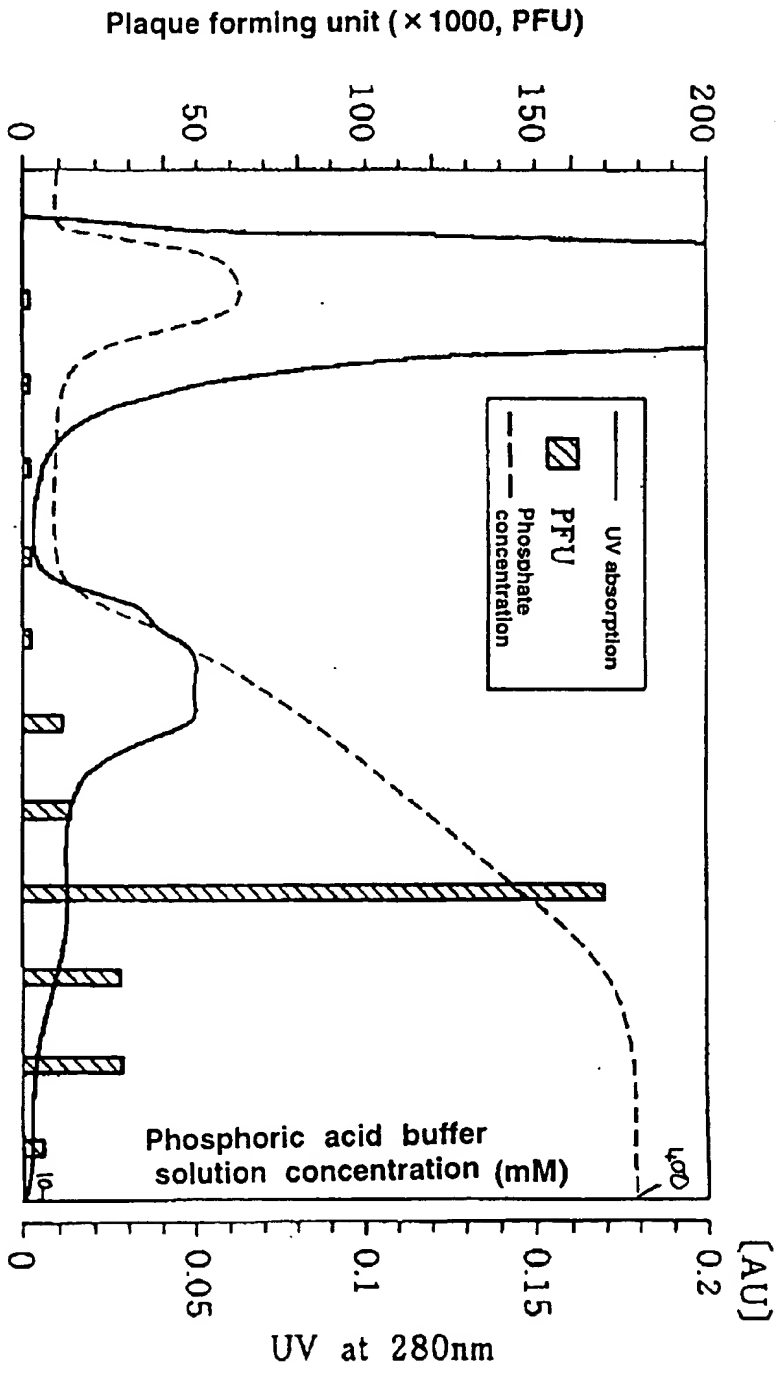
Plaque forming unit (×1000, PFU)
0
50
100
150
200
1
2
3
4
5
6
7
8
9
10
11
12
Fraction No.
UV absorption
PFU
Phosphate concentration
Phosphoric acid buffer solution concentration (mM)
400
10
0
0.05
0.1
0.15
0.2
[AU]
UV at 280nm

Figure 7

VIRUS OR VIRAL ANTIGEN PURIFICATION METHOD AND VACCINE PRODUCING METHOD

The present invention relates to a virus or viral antigen purification method and vaccine producing method, and in particular to a method for easily separating and purifying a virus or viral antigen from a culture solution containing the virus or viral antigen, and a vaccine producing method using the purification method.

Separation and purification of a virus or viral antigen (hereinafter, also referred to as "virus or the like") from a culture solution, culture medium and host cells are important steps in the fields of genetic engineering, clinical diagnosis and vaccine production.

Generally, in the culture solution or the like, a virus or viral antigen does not exist alone, but exists together with cell culture cells and impure proteins. Thus, it is necessary to separate and purify only a virus or viral antigen from the culture solution or the like.

Conventionally, separation and purification of a virus or viral antigen have been performed by means of an

ultra-centrifugal separating method or a density inclination centrifugal method or the like. However, since these methods require expensive and large-scale apparatus and complicated operations, work involving these methods is very troublesome.

An alternative method for extracting a virus employing column chromatography using hydroxyapatite has been proposed (Sumiaki Tsuru et al., Bio-Medical Materials and Engineering Vol. 1, pp. 1-5, 1991).

According to this method, an eluant of substantially pH 7 is used, to extract a virus or the like from a sample at a high extracting rate.

However, this method leads to an unsatisfactorily separation of the virus or the like from impure proteins. Therefore, when the extracted virus is used for producing a vaccine, ultra-centrifugal separation must be further carried out. Thus, there is a problem in that it is necessary to carry out a further purification process in order to obtain a virus of a high purity.

A further method for purifying a virus employing column chromatography in which silica is used as a solid carrier has been proposed. In this method, there is an advantage that a virus can be extracted by one step from a biological material such as a culture solution. However, since an eluant whose pH is high is used, activity of the virus is liable to be lowered. Therefore, in this method, it

is very difficult to efficiently purify the virus without lowering activation.

An object of the invention is to provide a virus or viral antigen purification method by which a virus or the like can be easily and quickly separated and purified from a culture solution or cell emulsified solution or the like containing the virus or viral antigen, without losing or lowering activation of the virus or viral antigen,

A further object of the invention is to produce a vaccine using said purification method.

According to one embodiment of the present invention there is provided a virus or viral antigen purification method comprising the steps of: contacting a sample containing a virus or viral antigen with hydroxyapatite as a solid carrier, said hydroxyapatite being sintered at between substantially 400 to 1300°C and capable of absorbing the virus or viral antigen; and eluting the virus or viral antigen absorbed by the solid carrier using an eluant within a neutral pH range.

Hydroxyapatite sintered at the above-mentioned temperature range has a porous structure having fine pore diameters and a porous rate that can exhibit excellent virus absorbing capacity.

In this connection, only a virus or viral antigen will be efficiently separated and purified from impure protein or the like which is coexisting with the virus or viral antigen

in the sample. Further, the hydroxyapatite which has been sintered at the above temperature range has an increased strength, so that the hydroxyapatite does not collapse even while having sufficient pores to absorb the virus or viral antigen. Furthermore, such hydroxyapatite is sufficiently durable against any pressure applied in the purification process by means of column chromatography.

By using an eluant within a neutral pH range, a virus whose activation is maintained can be obtained at a high purity. Here, the neutral range means a range of substantially 6 through 7.5 pH

Preferably, the hydroxyapatite is formed of spherical porous particles. In a preferred embodiment, the average particle diameter of the hydroxyapatite is 10 through 100μm. Conveniently, the hydroxyapatite is formed of porous particles.

Preferably, the eluant is a buffer solution.

Conveniently, the buffer solution is a phosphoric acid-based buffer solution.

In the present invention, it is preferred that the size of the virus or viral antigen is between substantially 10 through 500nm.

Preferably the virus or viral antigen is obtained from a living animal or cell culture cells. In a preferred embodiment, the virus or viral antigen is propagated in brain cells or neurons of mammals. Conveniently the virus or viral antigen may be propagated in

an egg. Preferably the virus is propagated in cell culture cells.

In the present invention, it is preferred that the virus is the Japanese encephalitis virus or an influenza virus.

Further, in the present invention, it is preferred that the virus or viral antigen is purified by means of column chromatography.

Furthermore, it is also preferred that the eluting speed in the eluting process is 0.05 through 2 ml/min.

According to a further embodiment of the present invention there is provided a method for producing a vaccine comprising: purifying the virus or viral antigen by means of the method of purification as set forth in any one of Claims 1 to 15; and a process for inactivating the virus or viral antigen purified in the purification process.

These and other objects, structures and advantages of the present invention will be apparent more clearly from the following description of the invention based on the examples.

Fig. 1 is a diagram showing the eluting pattern of the Japanese encephalitis virus from a mouse brain emulsion according to the virus or viral antigen purification method of the invention (hydroxyapatite was sintered at 400°C with a particle size of 40μm is used; the eluting speed was 0.5 ml/min).

Fig. 2 is a diagram showing the eluting pattern of the Japanese encephalitis virus according to the virus or viral antigen purification method of the invention.

Fig. 3 is a diagram showing the eluting pattern of the

Japanese encephalitis virus according to the virus or viral antigen purification method of the invention.

Fig. 4 is a diagram showing the eluting pattern of an influenza virus or viral antigen from a sample of infected egg according to the virus or viral antigen purification method of the invention (hydroxyapatite was sintered at 400°C; the eluting speed was 0.1 ml/min).

Fig. 5 is a diagram showing the eluting pattern of an influenza virus or viral antigen from a sample of infected egg according to the virus or viral antigen purification method of the invention (hydroxyapatite was sintered at 700°C; the eluting speed was 0.1 ml/min).

Fig.6 is a diagram showing the eluting pattern of an influenza virus or viral antigen from a sample of infected egg according to the virus or viral antigen purification method of the invention (hydroxyapatite was sintered at 1000°C; the eluting speed was 0.1 ml/min).

Fig. 7 is a diagram showing the eluting pattern of the Japanese encephalitis virus from a sample of infected mosquito cells according to the virus or viral antigen purification method of the invention (hydroxyapatite was sintered at 1000°C; the eluting speed was 0.5 ml/min).

"AU" in the Figures 1 to 7 means "arbitary units".

The present invention comprises a process in the which a sample containing a virus or viral antigen is contacted with hydroxyapatite sintered at between substantially 400 to 1300°C, wherein the hydroxyapatite is a solid carrier capable of absorbing the virus or viral antigen, and a process in that the virus or viral antigen absorbed by the solid carrier is eluted by using an eluant within a neutral pH range.

Here, the "neutral pH range" means a range of substantially pH 6 to pH 7.5. By using an eluant within this neutral pH range,

a virus whose activation is maintained can be obtained at a high purity.

In the invention, as a solid carrier, hydroxyapatite which is sintered at a sintering temperature of substantially 400 to 1300°C is used, and more preferably, hydroxyapatite sintered at between substantially 950 to 1050°C is used.

Hydroxyapatite sintered at such a temperature range has a porous structure having fine pore diameters and a porous rate that can exhibit excellent virus absorbing capacity. In this respect, a virus or viral antigen can be efficiently separated and purified from impure protein or the like which coexists with the virus or viral antigen in a sample. Further, a hydroxyapatite which has been sintered at the above temperature range has an increased strength, so that the hydroxyapatite does not collapse even while having sufficient pores to absorb the virus or viral antigen. Furthermore, such hydroxyapatite is sufficiently durable against any pressure applied in the purification process by means of column chromatography.

In a case where the sintering temperature of the hydroxyapatite is less than 400°C, the absorbing capacity of the hydroxyapatite becomes too high, thus leading to the case where elution of the virus or viral antigen in the eluting process described later becomes difficult, or impure protein is also absorbed. On the other hand, when the

sintering temperature is higher than 1300°C, the hydroxyapatite is apt to be heat-decomposed so that it becomes unavailable as a solid carrier.

Particularly, in hydroxyapatite sintered between substantially 950 to 1050°C, the number of pores to be formed is limited such that absorption of impure protein can be restricted.

Preferably, the hydroxyapatite used in this invention is formed of spherical porous particles. By using a porous structure the effective surface area per unit volume of the hydroxyapatite can be maximized, which makes it possible to improve the virus absorbing capacity.

Hydroxyapatite can be effectively filled into a chromatograph column at a high density, allowing the virus separation to be carried out in a stable manner. In addition, even though the hydroxyapatite is filled in the column at a high density, spaces are formed between the particles due to spherical shapes of the particles, which makes it possible to satisfactorily maintain liquid permeation of an eluant such as a buffer solution.

Preferably, the hydroxyapatite has an average particle diameter of 10 through 100μm, and more preferably, 40 through 80μm. In a case where the particle diameter is less than 10μm, it becomes difficult for the virus or viral antigen to pass through the spaces between the hydroxyapatite particles,

Accordingly, the amount of virus or viral antigen absorbed by the hydroxyapatite may be lowered. On the other hand, if the particle diameter exceeds 100μm, the particle spaces become too large, and the speed that the virus or viral antigen passes through the particle spaces increases, . makes it difficult for the virus or viral antigen to be absorbed by the hydroxyapatite. Also, if the particles are too large, the interstitial space between each particle (packed beads) become large. The void space between the packed beads in the column may induce the diffusion of the separated fractions (or bands). Accordingly, the resolution of the column is affected.

Furthermore, it is preferable that the hydroxyapatite is formed of porous particles. If the hydroxyapatite is formed of dense particles which have few pores the surface area per unit volume is small, which results in the case where a sufficient virus absorbing capacity cannot be obtained.

With regard to the porosity of the hydroxyapatite particle, 50 to 80% porosity is preferable and 65 to 75% is more preferable. If the porosity is too high, it is not possible to achieve sufficient absorptivity due to relatively small specific surface area, while if the porosity is too low, mechanical strength is liable to be lowered.

The spherical porous particles of the hydroxyapatite can be produced by the following methods. First, hydroxyapatite slurry obtained by a generally-known wet method is formed into particles by being directly spray-dried, thereby forming spherical porous particles. Alternatively, spherical porous particles can be formed by adding an additive such as a viscosity regulator, heat-decomposable organic compound particles or fiber or the like to the hydroxyapatite slurry, and then spray-drying. Next, the spherical porous particles

produced by either of the above methods are sintered at between substantially 400 to 1300°C to produce porous spherical particles.

The size of the virus or viral antigen to be purified is not particularly limited, but preferably, approximately 10 through 500nm. The virus or viral antigen within this range can be satisfactorily separated and purified by the method according to the present invention.

Preferably, examples of the virus to be purified include an influenza virus having a size of approximately 100 through 120nm or the Japanese encephalitis virus of approximately 40 through 50nm. Further, examples of the viral antigen include structural proteins of the influenza virus or Japanese encephalitis virus or the like.

In the present invention, it is possible to use the virus or viral antigen which is obtained by being propagated in a living animal or cell culture cells. More specifically, for example, a virus propagated in the brain cells or neurons of mammals, a virus propagated in an egg, and a virus propagated in cell culture cells, etc., can be preferably used. By propagating the virus in such a system, it is possible to obtain a large quantity of the virus constantly. Further more, in the case where cell culture cells are used for propagating the virus, the virus can be cultured in a condition of relatively few impurities, which result in a relatively easy purification of the virus.

As for the method for contacting the solid carrier formed

from hydroxyapatite with the virus or viral antigen in the contacting process, various methods can be selectively used depending on the formation of the solid carrier to be used and the volume of the sample and the like. For example, a batch method could be used whereby hydroxyapatite is thrown into a sample to absorb the virus, or a method whereby a sample is added into a column filled with hydroxyapatite can be used.

Next, the virus absorbed by hydroxyapatite as a solid carrier is eluted. In a method of the present invention, an eluant within a neutral range is used to separate and collect a desired virus from the solid carrier in the eluting process. For example, in the case of column chromatography, fractions containing the desired virus are separately extracted.

By using such an eluant in the neutral range, the virus can be easily eluted. In addition, lowering of activation or denaturation of the virus can be suppressed.

An eluant in such a neutral pH range, for example, a buffer solution having a buffering capacity in a range of pH6 through pH7.5 can be used. Examples of such a buffer solution include a phosphoric acid-based buffer solution, a sodium acetate-acetic acid-based buffer solution, a sodium acetate-hydrochloric acid-based buffer solution or trisaminomethane-based buffer solution or the like. Among these solutions, the phosphoric acid-based buffer solution is particularly preferable. The phosphoric acid-based buffer

solution can be used as a buffer solution that exhibits a buffer action at substantially pH7, whereby separation and purification of the virus can be performed while maintaining the activation of the virus in stable condition.

In this connection, it is preferred that, before proceeding to the eluting process from the absorbing process of the virus by hydroxyapatite, the hydroxyapatite is washed several times using an eluant with a low concentration of salt in order to remove as many as possible of the impurities which are trapped in the column and are not absorbed by the hydroxyapatite. Examples of such impurities include the crushed matter of the culture cells, parts of the virus particles, and impure protein and the like which exist in the sample.

Elution of the virus can also be performed by increasing the concentration of salt in the buffer solution, for example. The concentration of salt in the buffer solution can be increased in accordance with either a straight inclination or curved inclination. According to this method, a substance having low absorptive such as protein is first eluted from the hydroxyapatite. This means that it is possible to selectively separate a desired virus, which has high absorption capacity with respect to the hydroxyapatite, from the hydroxyapatite.

A batch method or column

chromatography or the like can be used to purify the virus or viral antigen. Among these methods, the column chromatography is particularly preferable, because the virus or viral antigen can be purified at a high extracting rate with high purity, and reproductivity thereof is excellent.

In a case where the virus or viral antigen is separated by column chromatography, the velocity of the flowing fluid in the eluting process is properly selected in accordance with the capacity of the sample and capacity of the column. In this case, it is preferable that the velocity is 0.05 through 2ml/min, and more preferably, 0.1 through 1ml/min.

If the velocity of the fluid is too slow, it takes a relatively long time to carry out the elution of the virus, which results in lowering the activity of the virus. On the other hand, if the velocity of the fluid is too fast, reproductivity of the separating efficiency of the virus may be deteriorated.

Further, the vaccine producing method of the invention is characterized by carrying out a virus or viral antigen purifying process according to the present invention, and a process for inactivating the virus or viral antigen purified by said purifying process. In this connection, since the virus ir viral antigen is purified at a high purity, a danger of contamination due to other microbes can be reduced, thereby allowing a highly

safe vaccine to be produced.

In the process for inactivating the virus or viral antigen purified by the method of the present invention, inactivation can be performed by various methods in accordance with the type of vaccine to be produced.

The virus or viral antigen purifying method and vaccine producing method of the present invention are described in the above. However, the present invention is not limited thereto. For example, for refining of the virus or viral antigen, it is possible to use a solid carrier in which a ligand having biological specific interaction with a target virus are carried on the surface of hydroxyapatite particles.

Hereinbelow, detailed examples of the present invention will be described.

Example 1

Hydroxyapatite slurry obtained by a wet method (molar ration of Ca/P is 1.67) was formed into particles by being directly spray-dried, thereby producing spherical secondary particles of hydroxyapatite. Next, the spherical secondary particles were sintered at the respective temperatures of 400°C, 700°C, and 1000°C, and then the sintered spherical porous particles were classified to obtain

hydroxyapatite particles having a particle diameter of 40μm. By using thus obtained hydroxyapatite particles as a solid carrier, the Japanese encephalitis virus was purified by column chromatography.

Japanese encephalitis virus was propagated in a mouse's brain, and then the brain was extracted, and a solution of the emulsified brain (Japanese encephalitis virus infected mouse brain emulsion) was used as the sample.

0.45g of hydroxyapatite particles obtained by being sintered at 400°C, 0.45g hydroxyapatite particles obtained by being sintered at 700°C of and 0.9g of hydroxyapatite obtained by being sintered at 1000°C were placed onto separate filters with pores having a size of 35μm installed in a syringe having an inner diameter of 8mm and a height of 20mm. Into each column thus prepared, 0.1ml of the Japanese encephalitis virus infected mouse brain emulsion was added.

Next, a buffer solution of phosphoric acid of pH7 was used as an eluant to elute the Japanese encephalitis virus. The buffer solution of phosphoric acid having a linear-gradient of a phosphoric acid concentration of 10mM through 400mM was supplied into the column at a velocity of fluid of 0.5ml/min, whereby elution was performed.

The solution passed through the column was extracted by each fraction of 1ml, and biological activity of the Japanese encephalitis virus in each fraction was calculated in

accordance with a plaque assay in Vero cells (obtained from the kidney of the African Green Monkey). Also, protein content in each fraction was inspected by means of the UV absorbing spectrum (280nm). The results are shown in Fig. 1 to Fig. 3.

Furthermore, protein in each fraction was detected by SDS-PAGE.

As clearly understood from Fig. 1 to Fig. 3 and the results of the SDS-PAGE (not illustrated), in all cases, most of the impure protein was not absorbed by hydroxyapatite, or washed out by the buffer solution of phosphoric acid of a low concentration. On the other hand, the Japanese encephalitis virus was securely absorbed by the hydroxyapatite, and washed out by the buffer solution of phosphoric acid of a high concentration, thereby being satisfactorily separated from the impure protein.

Also, the Japanese encephalitis virus collected in the fractions No. 8 through No. 10 maintained viral activity, while having a high purity and a high collecting rate.

Example 2

As is the same with the Example 1, an influenza virus (viral antigen) was purifiedby column chromatography using hydroxyapatite particles having a particle diameter of 40μm obtained by being sintered at 400°C, 700°C, and 1000°C, respectively.

The influenza virus was propagated in a chicken embryo egg which was subsequently processed for use as a sample.

The same buffer solution of phosphoric acid as in Example 1 was supplied at a velocity of fluid of 0.1ml/min, and the solution passed through the column was collected in fractionsof 2ml, and the biological activity of the influenza virus in each fraction was calculated by the plaque assay in MDCK (Madin-Darby Canine Kidney cells).

Also, the protein content in each fraction was inspected by means of a UV absorbing spectrum (280nm).

Furthermore, hemagglutination assay was performed for each fraction, and HA protein which was one of the viral antigens was detected.

The results are shown in Fig. 4 through Fig. 6.

As clearly understood from Fig. 4 through Fig. 6, in all cases, most of the impure protein was not absorbed by hydroxyapatite, or washed out by the buffer solution of phosphoric acid of a low concentration. On the other hand, the influenza virus (viral antigen) was securely absorbed by the hydroxyapatite, and washed out by the buffer solution of phosphoric acid of a high concentration, thereby being satisfactorily separated from impure protein.

Also, the influenza virus collected in the fractions No. 4 and No. 5 had a high purity and high

collecting rate, and it was found that such an influenza virus had biological activity.

Furthermore, the results of the hemagglutination assay were also the same as the results of the plaque assay.

Example 3

The Japanese encephalitis virus was purified in the same way as in Example 1 except that a sample comprising the top clear layer of culture cells obtained by propagating Japanese encephalitis virus in C6/36 (mosquito's cells) was used.

The results in respect of the hydroxyapatite sintered at 1000°C are shown in Figure 7; similar results were obtained when hydroxyapatite sintered at 400°C and 700°C, was used.

As clearly understood from Fig. 7 and the results of the SDS-PAGE (not illustrated), in all cases, most of the impure protein was not absorbed by the hydroxyapatite, or washed out by a buffer solution of phosphoric acid of a low concentration. On the other hand, the Japanese encephalitis virus was securely absorbed by the hydroxyapatite and washed out by a buffer solution of phosphoric acid of a high concentration, thereby being satisfactorily separated from the impure protein.

The Japanese encephalitis virus collected in the fraction No. 9 had a high purity and a high collection rate, and it was found that such a Japanese encephalitis virus had biological activity.

Furthermore, in comparison with the results of Example 1, the eluting pattern of the virus was sharp, and thereby it was found that the separating capacity was excellent.

Also, when the virus was purified in the same way as in Examples 1 through 3 using a particle diameter of 80μm of hydroxyapatite obtained at the respective sintering temperatures, the same results as those in Examples 1 through 3 were obtained.

As described above, according to the virus or viral antigen purification method of the invention, a highly pure virus or viral antigen can be efficiently obtained by simple operations.

Furthermore, since the virus or viral antigen separated and purified by the method of the present invention satisfactorily maintains the biological activity, it is possible to produce an effective and safe vaccine.

Finally, it is to be understood that many changes and additions may be made to the embodiments described above without departing from the scope and spirit of the invention as defined in the following claims.

Further, it is also to be understood that the present disclosure relates to subject matter contained in Japanese Patent Application No. 11-073915 (filed on March 18, 1999) which is expressly incorporated herein by reference in its entirely.

CLAIMS :-

1. A virus or viral antigen purification method, comprising the steps of:

contacting a sample containing a virus or viral antigen with hydroxyapatite as a solid carrier, said hydroxyapatite being sintered at between substantially 400 to 1300°C and capable of absorbing the virus or viral antigen; and

eluting the virus or viral antigen absorbed by the solid carrier using an eluant within a neutral pH range.

2. A virus or viral antigen purification method according to Claim 1, wherein the hydroxyapatite is formed of spherical porous particles.

3. A virus or viral antigen purification method according to either Claim 1 or 2, wherein the average particle diameter of the hydroxyapatite is 10 through 100μm.

4. A virus or viral antigen purification method according to any preceding claim, wherein the hydroxyapatite is formed of porous particles.

5. A virus or viral purification method according to any preceding claim, wherein the eluant is a buffer solution.

6. A virus or viral antigen purification method according to Claim 5, wherein the buffer solution is a phosphoric acid-based buffer solution.

7. A virus or viral antigen purification method according to any preceding claim, wherein the size of the virus or viral antigen is between substantially 10 to 500nm.

8. A virus or viral antigen purification method according to any preceding claim, wherein the virus or viral antigen is obtained from a living animal or cell culture cells.

9. A virus or viral antigen purification method according to any preceding claim, wherein the virus or viral antigen is propagated in brain cells or neurons of mammals.

10. A virus or viral antigen purification method according to any preceding claim, wherein the virus or viral antigen is propagated in an egg.

11. A virus or viral antigen purification method according to any preceding claim, wherein the virus is propagated in cell culture cells.

12. A virus or viral antigen purification method according to any preceding claim, wherein the virus is the Japanese encephalitis virus.

13. A virus or viral antigen purification method wherein the virus is an influenza virus.

14. A virus or viral antigen purification method as set forth in Claim 1, wherein the virus or viral antigen is refined by means of column chromatography.

15. A virus or viral antigen purification method as set forth in Claim 1, wherein the eluting speed in the eluting process is 0.05 through 2 ml/min.

16. A virus or viral antigen purification method as set forth in Claim 1, wherein the "neutral range" is a range of

approximately pH 6 through pH 7.5.

17. A method for producing a vaccine comprising:

purifying the virus or viral antigen by means of the method of purification as set forth in any one of the Claims 1 to 16; and

a process for inactivating the virus or viral antigen purified in the purification process.

Application No: GB 0006716.5 **Examiner:** Dr Patrick Purcell
Claims searched: 1-17 **Date of search:** 8 August 2000

Patents Act 1977
Search Report under Section 17

Databases searched:

UK Patent Office collections, including GB, EP, WO & US patent specifications, in:

UK Cl (Ed.R):

Int Cl (Ed.7):

Other: ONLINE: EPODOC, WPI, JAPIO, BIOSIS, CAPLUS

Documents considered to be relevant:

Category	Identity of document and relevant passage	Relevant to claims
P, X	WO 00/09671 A1 (MERCK & CO, INC) see whole document, especially page 3, line 9-page 5, line 9	1-8, 14-17
Y	EP 0337123 A1 (KANTO KAGAKU KABUSHIKI KAISHA) see whole document, especially page 3, lines 29-39, page 7, lines 5-12	1-16
X	US/EG Bulletin 2156, BIO-RAD Laboratories, 1996, P Gagnon et al, "Ceramic Hydroxyapatite- A new Dimension in ...", see pages 1-2	1 at least
X, Y	Biochim Biophys Acta, 1982, 165-173, M Yamaki et al, "Circular dichroism and biomedical properties ...", especially results	X: 1 at least Y: 1-16
X, Y	J Virology, 1980, 35, 592-602, J Luka et al, "Purification and biochemical characterization of...", especially 2nd column page 594 and Fig. 1	X: 1 at least Y: 1-16
X	LIT611 Rev C, Macro-Prep^R Ceramic Hydroxyapatite Instruction Manual, BIO-RAD Laboratories, see pages 1-3, 12-13, 21 and Table 1	1 at least

X	Document indicating lack of novelty or inventive step	A	Document indicating technological background and/or state of the art.
Y	Document indicating lack of inventive step if combined with one or more other documents of same category.	P	Document published on or after the declared priority date but before the filing date of this invention.
&	Member of the same patent family	E	Patent document published on or after, but with priority date earlier than, the filing date of this application.